Turkish Journal of Botany

Volume 36 | Number 4 Article 8

1-1-2012

The relationship between the antioxidant system and phycocyanin production in Spirulina maxima with respect to nitrate concentration

RAZİYE ÖZTÜRK ÜREK

LEMAN TARHAN

TÜBİTAK

Research Article

Turk J Bot
36 (2012): 369-377
© TÜBİTAK
doi:10.3906/bot-1106-1

The relationship between the antioxidant system and phycocyanin production in *Spirulina maxima* with respect to nitrate concentration

Raziye ÖZTÜRK ÜREK, Leman TARHAN*

Biochemistry Division, Chemistry Department, Science Faculty, Dokuz Eylül University,
35160 Buca, İzmir - TURKEY

Received: 01.06.2011
Accepted: 09.12.2011

Abstract: The relationship among antioxidant enzyme activities, such as iron-superoxide dismutase (FeSOD) and ascorbate-dependent peroxidase (AsA-POD), and phycocyanin, chlorophyll, total carotenoid, ascorbate contents, and lipid peroxidation (LPO) levels were investigated in *Spirulina maxima* SAG 84.79 in nitrate limited (10 mM), control (30 mM), and supplemented (50 mM) conditions. In the present study, extracellular nitrate and intracellular iron, magnesium, and copper levels were also investigated. The highest FeSOD and AsA-POD activities and chlorophyll content and the lowest LPO levels were found in control conditions on day 13: 21.9 ± 1.5, 5.7 ± 0.3 IU mg^{-1}, 16.2 ± 0.5 mg g^{-1}, and 0.322 ± 0.01 nmol malondialdehyde g^{-1} wet weight, respectively. The highest phycocyanin content was determined in nitrate supplemented conditions on day 13, at 190.68 ± 9.3 mg g^{-1}. The similar LPO levels observed in supplemented (50 mM nitrate) and control (30 mM) conditions showed that phycocyanin demonstrated effective protection of *S. maxima* membranes. The data revealed that its response against nitrate exposure was related to the antioxidant enzymes and compounds in the antioxidant defence system of *S. maxima*.

Key words: *Spirulina*, superoxide dismutase, peroxidase, phycocyanin, lipid peroxidation

Spirulina maxima'da nitrat konsantrasyonuna bağımlı antioksidan sistem ve fikosiyanin üretimi arasındaki ilişki

Özet: Demir-süperoksit dismutaz (FeSOD) ve askorbat-bağımlı peroksidaz (AsA-POD) gibi antioksidan enzim aktiviteleri ve fikosiyanin, klorofil, total karotenoid, askorbat içerikleri ve ayrıca lipid peroksidasyon (LPO) düzeyleri arasındaki ilişki, nitrat sınırlı (10 mM), kontrol (30 mM), katkılı (50 mM) koşullara bağlı *Spirulina maxima* SAG 84.79 türünde incelendi. Bu çalışmada, hücre dışı nitrat ve hücre içi demir, magnezyum, bakır düzeyleri de araştırıldı. En yüksek FeSOD ve AsA-POD aktiviteleri, klorofil içeriği ve en düşük LPO düzeyleri, kontrol koşulunda 13. günde sırasıyla 21,9 ± 1,5, 5,7 ± 0,3 IU mg^{-1}, 16,2 ± 0,5 mg g^{-1} ve 0,322 ± 0,01 nmol malondialdehid g^{-1} yaş ağırlık olarak belirlendi. Ayrıca, en yüksek fikosiyanin içeriği, nitrat katkılı koşulda 13. günde 190,68 ± 9,3 mg g^{-1} olarak saptandı. 50 mM nitrat varlığında kontrolle benzer LPO düzeyleri, fikosiyaninin *S. maxima* membranlarını etkin koruduğunu gösterdi. Elde edilen veriler, nitrata karşı tepkinin, *S. maxima* antioksidan savunma sistemindeki antioksidan enzimler ve bileşenlerle ilişkili olduğunu açığa çıkardı.

Anahtar sözcükler: *Spirulina*, süperoksit dismutaz, peroksidaz, fikosiyanin, lipid peroksidasyonu

* E-mail: leman.tarhan@deu.edu.tr

Introduction

Cyanobacteria form a diverse group of bacteria that use oxygenic photosynthesis to produce energy from sunlight. Cyanobacteria are the first organisms that produced molecular oxygen. Like cyanobacteria, in aerobic organisms, reactive oxygen species (ROS) are inevitably formed. An excess of ROS in cellular components, namely hydrogen peroxide, superoxide anion, and hydroxyl radicals, are known to cause damage to membranes, DNA, and proteins (Mittler, 2002; Latifi et al., 2009). Under optimal conditions, the balance between ROS formation and consumption is tightly controlled by an array of antioxidant enzymes such as superoxide dismutase (SOD), peroxidases (PODs), glutathione reductase, and redox metabolites like ascorbate (AsA) and glutathione (Noctor & Foyer, 1998; Möller, 2001; Candan & Tarhan, 2005). The efficient destruction of ROS requires the action of several antioxidant enzymes. The respiratory electron transport and photosystem I in photosynthetic organisms contribute to the formation of superoxide radicals (Latifi et al., 2009). Superoxide anion radical is rapidly converted to H_2O_2 by the action of SOD (Öztürk-Ürek et al., 2001). H_2O_2 can be decomposed by catalase (CAT) as well as AsA-POD enzymes. AsA-POD uses 2 molecules of AsA to reduce H_2O_2 to water (Terzi et al., 2010). In addition, AsA is an important metabolite for most living organisms and well known for its antioxidant properties. When the balance between antioxidant system and ROS is impaired, the lipid peroxidation (LPO) process occurs. LPO is determined by the peroxide-deforming free radical mechanism and the peroxide-removing system.

Chlorophyll and carotenoids are naturally occurring pigments present in photosynthetic plants, including algae and cyanobacteria. The other cyanobacterial pigments, phycocyanins, have been studied due to their involvement as major accessory pigments in photosynthesis. They function by absorbing light in regions of low chlorophyll absorption and then participating in highly efficient exciton migration (Vonshak, 1997).

Spirulina sp. deserves special attention both as a source of single cell protein and because of its nutraceutical properties such as vitamins, minerals, proteins, and polyunsaturated fatty acids (Vonshak, 1997). *Spirulina maxima* Geitler is a planktonic filamentous cyanobacterium that forms massive populations in tropical and subtropical bodies of water that have high levels of carbonate and bicarbonate and alkaline pH values of up to 11. Nitrogen is required for the synthesis of amino acids, which make up proteins and other cellular components (Yang et al., 2010). However, in some cyanobacteria, the influence of nitrogen is marked in eukaryotic algae. Both the kind and the quantity of the nitrogen source in the culture medium can influence the growth and/or composition of *Spirulina* sp. biomass (Piorreck et al., 1984; Costa et al., 2001). Although the influence of growth conditions on the chemical composition of *S. maxima* has been investigated by many researchers, research on the relationship between the antioxidant enzyme system, phycocyanin content, and LPO level is limited. The aim of this study was to investigate the relationship between the antioxidant enzyme systems such as SOD, AsA-POD, and phycocyanin, chlorophyll, total carotenoid, AsA contents, LPO levels, and metal levels in *S. maxima* with respect to nitrate limited (10 mM), supplemented (50 mM), and control (30 mM) conditions.

Materials and methods

Organism and culture conditions

The cyanobacterium *Spirulina maxima* SAG 84.79 used in this study was obtained from the algae culture collection of the University of Gottingen, Germany. Inoculums of the *S. maxima* were maintained in Zarrouk's medium (Zarrouk, 1966). A carbonate-bicarbonate buffer maintained a pH of 9.5 ± 0.2. No adjustments were made throughout the experiments. All reagents were of analytical grade. *S. maxima* was cultivated in batch cultures containing 650 mL of medium at 28 °C. The control concentration of 30 mM $NaNO_3$ was chosen because this was the level present in Zarrouk's medium. In growth conditions of *S. maxima*, 10 mM (limited) and 50 mM (supplemented) $NaNO_3$ was used as a nitrogen source. Culture was inoculated to an initial optical density of ca. 0.2. The cultures were mixed and bubbled continuously using filtered air. Illumination at 2500 lux (30 mmol photon m^{-2} s^{-1}) light intensity was also provided continuously by white fluorescent lamps. The light intensity was measured by a digital light meter (Luxtron LX-101).

Preparation of crude extract

The cells were harvested periodically by centrifugation (4000 × *g*, 10 min, 4 °C), washed with cold distilled water following a potassium phosphate buffer (20 mM, pH 7.4) and resuspended in the same buffer including polypropylene glycol 1200 in a volume equal to 1.5 times its weight. A 600 µL cell suspension was ground in 1.5-mL plastic vials with 0.5 g of glass beads (0.5 mm ø) in a mixer-mill for 5 min. Cell debris was removed by centrifugation at 16,600 × *g*, 4 °C for 15 min. The supernatant was used for the assays. All experiments (until the enzyme determination) were done at 0 °C to 4 °C.

Assay methods in crude extract

SOD activity was measured in crude extract by the method of Crosti et al. (1987) based on the inhibitory effect of SOD on the spontaneous autoxidation of 6-hydroxydopamine (6-OHDA) at 490 nm and 25 °C. SOD activity assays were carried out by adding the amount of enzyme solution required to halve the initial absorbance value of 6-OHDA autoxidation at 90 s. One international unit is defined as the amount of SOD required to inhibit the initial rate of 6-OHDA autoxidation by 50%. To determine the type of SOD in *S. maxima*, assays were performed with 5 mM NaCN and 5 mM H_2O_2. The activity was present with cyanide, indicating that it does not represent CuZnSOD, which is inhibited by cyanide. Hydrogen peroxide can be used to distinguish FeSOD and MnSOD because only FeSOD is inactivated. The SOD activity of *S. maxima* was inactivated with H_2O_2. Therefore, *S. maxima* SOD was detected as FeSOD.

The activity of AsA-POD was measured according to Nakano and Asada (1981) by monitoring the rate of AsA oxidation at 290 nm ($e = 2.8\ mM^{-1}\ cm^{-1}$). The reaction mixture contained 25 mM phosphate buffer (pH 7.0), 0.1 mM EDTA, 1 mM H_2O_2, 0.25 mM AsA, and the enzyme sample at 25 °C. No change in absorption was found in the absence of AsA in the test medium. One international unit of AsA-POD activity represents the amount of enzyme that oxidises 1 µmol of AsA per minute at 25 °C.

Analytical methods

Phycocyanin was extracted in 2.5 mM phosphate buffer (pH 7.0) after freezing and thawing and the content was determined by the method of Benett and Bogard (1973). Contents of chlorophyll and total carotenoid were measured as described by Lichtenthaler and Wellburn (1983) after extraction with 80% acetone. The protein content was assayed by the method of Bradford using bovine serum albumin as standard (Bradford, 1976). Ascorbic acid analysis of *S. maxima* was performed in HPLC. Next, 0.5 mL of 5% aqueous dithiothreitol and 3.5 mL of 1% aqueous meta-phosphoric acid were added to the samples of 1 mL (Kurilich et al., 1999). The samples were filtered (0.45 mm) and injected (10 mL) onto a heated (30 °C) Supelcogel C610H column (30 cm × 7.8 mm) that was protected with a Supelcogel C610H guard column (5 cm × 4.6 mm) (Supelco, Inc, Bellefonte, PA, USA). The HPLC system had a UV detector set at 210 nm (Hewlett-Packard, Palo Alto, CA, USA). The mobile phase was 0.1% phosphoric acid. Flow rate was 0.5 mL min^{-1} and run time was 40 min per sample. Quantification was obtained using external L-ascorbic acid standards prepared in 5% dithiothreitol and 1% meta-phosphoric acid. Phycocyanin, chlorophyll, total carotenoid, and AsA contents were calculated in mg g^{-1} fresh weight.

LPO was estimated based on thiobarbituric acid (TBA) reactivity. Samples were evaluated for malondialdehyde (MDA) production using a spectrophotometric assay for TBA (Buege and Aust, 1976). The extinction coefficient at 532 nm of 153 000 $M^{-1}\ cm^{-1}$ for the chromophore was used to calculate the MDA-like TBA produced.

The nitrate level in the extracellular medium was determined on alternate days, using an Orion 9707 nitrate ion selective electrode connected to an Orion 720-Aplus potentiometer. The conductivity values obtained were correlated to the actual ammonia concentrations in the cultivations through calibration curves prepared on each analysis day, using solutions of known concentrations of ammonia (Leduy & Samson, 1982).

The levels of some metals were measured by inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 2100DV, Perkin Elmer, USA). *S. maxima* cells (1 g wet weight) were digested in 2 mL of nitric acid followed by 2 mL of perchloric acid. All glassware and apparatus were washed with 0.1 M nitric acid and ultra-pure water before use.

Statistical analysis

The Tukey test was used for statistical significance analyses. The values were the mean of 3 separate experiments. Comparisons were also made with Pearson's correlation.

Results

In the growth medium of *S. maxima*, the consumption rate of nitrate depending on time increased with increasing nitrate concentrations (Figure 1). On day 8, the decreases in extracellular nitrate concentrations in growth media containing 10 (limited), 30 (control), and 50 mM (supplemented) nitrate were determined to be 6.1 ± 0.2, 16 ± 0.7, and 29.1 ± 1.1 mM, respectively. The levels of nitrate for all investigated conditions were decreased in the following days of the incubation period.

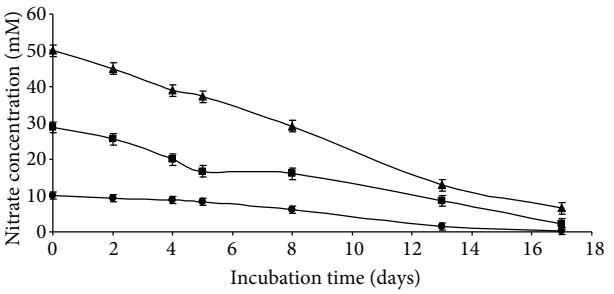

Figure 1. Variations in extracellular nitrate concentrations in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

Variations of intracellular iron, magnesium, and copper levels

Investigated intracellular metal levels of *S. maxima* depending on both testing nitrate concentrations and incubation time are presented in the Table. Uptakes of iron into the *S. maxima* cell increased up to day 13, after which they remained fairly constant. Magnesium uptake by *S. maxima* was constantly enhanced for all of the investigated conditions. Although the intracellular copper levels of the control group were increased with respect to the incubation period, these levels were significantly decreased from that of day 13 ($P < 0.05$) in the supplemented group.

Effects of nitrate concentration on the SOD and AsA-POD activities in *Spirulina maxima*

As can be seen from Figure 2, FeSOD activity, one of the antioxidant enzymes of *S. maxima*, reached a maximum on day 13. The increases in FeSOD activities between days 8 and 13 of the incubation period with increasing nitrate concentrations were determined as 2.24-, 2.14-, and 1.12-fold, respectively ($P < 0.05$). FeSOD activity was induced to a greater degree in limited nitrate conditions compared with the control and supplemented conditions. The highest FeSOD activity of *S. maxima* was determined in the control conditions at 21.9 ± 1.5 IU mg^{-1}. After day 13, FeSOD activities decreased with prolongation of the incubation period. The decrease in this activity seen in limited nitrate was higher than that of the control and supplemented conditions, which were similar.

The AsA-POD activities determined in *S. maxima*, which were dependent upon both the nitrate

Table. Variations of iron, magnesium, and copper levels in *S. maxima* depending the incubation period in medium containing 10, 30, or 50 mM concentrations of sodium nitrate. The values are the mean ± SEM of 3 separate experiments.

Metals	Incubation time (d)	$[NaNO_3]$ (mM)		
		10	30	50
Iron levels (mM)	8	196.96 ± 7.9	296.15 ± 8.3	257.12 ± 8.1
	13	372.96 ± 9.4	400.54 ± 9.5	340.91 ± 9.1
	17	363.35 ± 9.3	396.06 ± 9.4	323.18 ± 9.1
Magnesium levels (mM)	8	874.89 ± 11.1	1961.73 ± 19.4	1795.88 ± 17.3
	13	1230.45 ± 12.3	2758.02 ± 27.1	2497.94 ± 25.2
	17	2388.48 ± 23.7	4042.38 ± 40.1	3020.57 ± 30.6
Copper levels (mM)	8	2.36 ± 0.01	2.05 ± 0.01	1.89 ± 0.01
	13	2.36 ± 0.01	2.52 ± 0.01	2.05 ± 0.01
	17	2.67 ± 0.01	2.99 ± 0.01	1.42 ± 0.01

concentration and incubation time, are presented in Figure 3. Depending on the amount of nitrate present in the growth conditions, AsA-POD activities in *S. maxima* increased between days 8 and 13 of the incubation period and then decreased. Similar to FeSOD activity, the highest AsA-POD activity was determined in the control conditions on day 13, at 5.7 ± 0.3 IU mg^{-1}. In addition, our findings did not establish any CAT activity, using H_2O_2 as a substrate, in this cyanobacterium (not shown).

Effects of nitrate concentration on the phycocyanin, chlorophyll, total carotenoid, and AsA contents

As can be seen in Figure 4, the highest phycocyanin content was determined in the supplemented conditions on day 13, at 190.68 ± 9.3 mg g^{-1}. However, the phycocyanin content for the limited nitrate conditions, when compared to the control and supplemented conditions, was significantly decreased ($P < 0.05$).

The chlorophyll contents of *S. maxima* with respect to nitrate concentrations are depicted in Figure 5. Between days 8 and 13, chlorophyll contents for the control and supplemented conditions significantly increased by 50.9% and 34.2%, respectively ($P < 0.05$). The chlorophyll contents of those in limited conditions showed only insignificant variations, depending on the investigated incubation period ($P > 0.05$).

As shown in Figure 6, the highest total carotenoid content was determined in the supplemented conditions on day 17, at 5.2 ± 0.2 mg g^{-1}. There was no significant difference on day 8 for the total

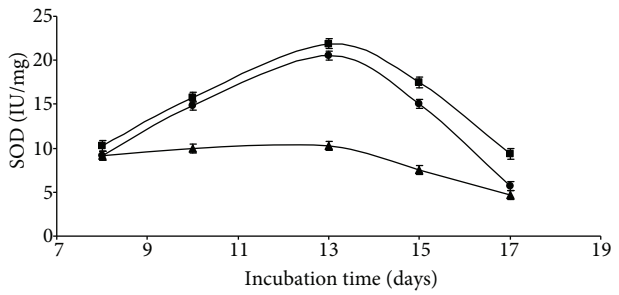

Figure 2. Variations in SOD activity in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

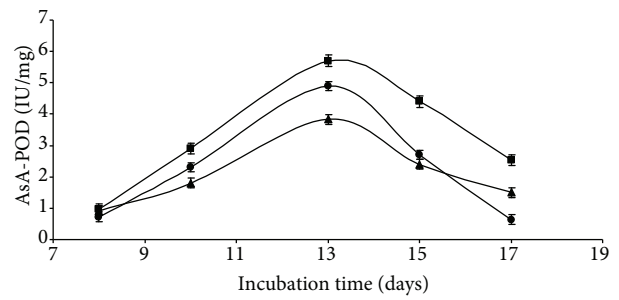

Figure 3. Variations in AsA-POD activity in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

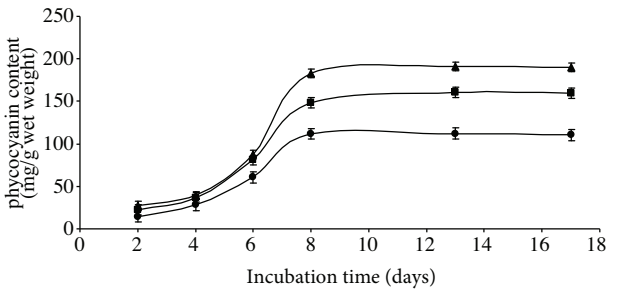

Figure 4. Variations in phycocyanin levels in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

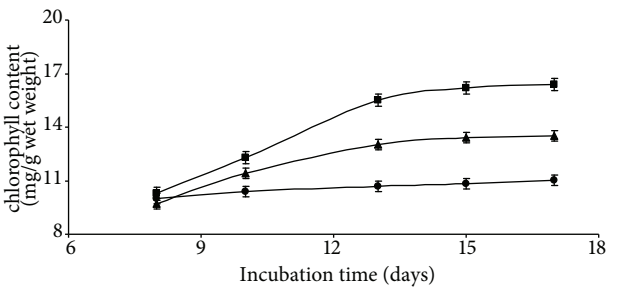

Figure 5. Variations in chlorophyll levels in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

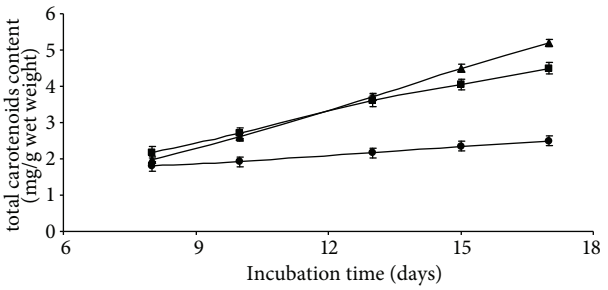

Figure 6. Variations in total carotenoid levels in *S. maxima* depending the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

carotenoid contents of *S. maxima* grown in all of the nitrate concentrations tested ($P > 0.05$). After day 8, the total carotenoid content of those under nitrate limited conditions was significantly decreased in comparison to those in the control and supplemented growth media ($P < 0.01$).

As can be seen in Figure 7, AsA content significantly increased for the limited and control conditions between days 13 and 17 ($P < 0.05$). There was also an insignificant increase in the nitrate supplemented conditions ($P > 0.05$). AsA contents on day 17, when compared with those from day 13, were significantly increased, by 128.4% for limited nitrate conditions and by 63.7% for control conditions ($P < 0.01$). The highest AsA content determined on day 17 for control conditions was 8.3 ± 0.2 mg g^{-1}.

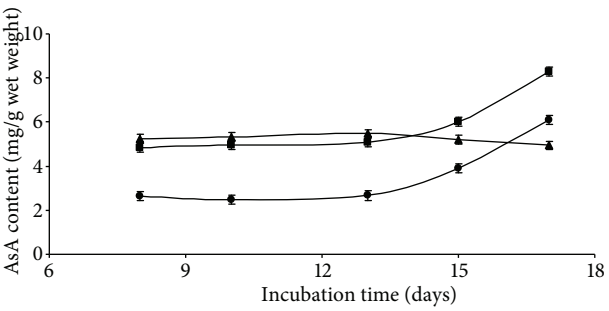

Figure 7. Variations in AsA levels in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

Variations in LPO level

Cyanobacterial membranes have a high content of polyunsaturated fatty acids, which are easily oxidised to form lipid peroxides. To evaluate the membrane damage caused by ROS, LPO levels in *S. maxima* were determined depending on both the nitrate concentration provided and incubation time. As shown in Figure 8, the levels of LPO in control conditions decreased significantly between days 8 and 13, by 34.55% ($P < 0.01$). The minimum LPO level on day 13 was determined in the control conditions as 0.322 ± 0.01 nmol MDA g^{-1} wet weight. Under the nitrate supplemented conditions, the levels of LPO significantly decreased between days 8 and 13 ($P < 0.01$) and then increased in the following incubation days. However, LPO levels in the nitrate limited conditions significantly decreased ($P < 0.05$) on day 13 and thereafter remained fairly constant.

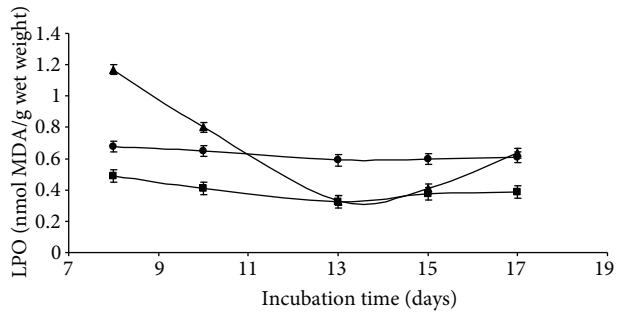

Figure 8. Variations in LPO levels in *S. maxima* depending on the incubation period in medium containing 10 mM (-●-), 30 mM (-■-), or 50 mM (-▲-) sodium nitrate concentrations. The values are the mean ± SEM of 3 separate experiments.

Discussion

Cyanobacterium cultivation generally includes nitrates added as a nitrogen source when grown as batch cultures (Rai & Tiwari, 2001; Moore et al., 2002). The use of nitrate in cultivation causes an increase in the biomass production as well as in pigment content such as chlorophyll. The metabolism of nitrate, reducing nitrate to nitrite and then to ammonium and, by subsequent ammonium incorporation, to carbon skeletons yielding amino acids, has been demonstrated for *S. platensis* and *Synechococcus* sp. WH 7803 (Boussiba, 1989; Chadd et al., 1996). The aim of the present study was to investigate the influence of different nitrate concentrations in *S.*

maxima growth media on variations in antioxidant systems as well as on metal uptake. Extracellular nitrate levels in *S. maxima* growth medium in the range of 10-50 mM decreased linearly depending on the incubation period. The consumption rate also showed positive correlation with the increase in nitrate concentrations ($r = 0.945$, $P < 0.01$). This situation shows that there is nothing in the *S. maxima* cell membrane to prevent nitrate transport in the investigated nitrate concentrations.

In the present study, variations in FeSOD and AsA-POD enzyme activities were examined with regard to nitrate concentrations. Typically, cyanobacteria contain MnSOD, FeSOD, or NiSOD, and rarely CuZnSOD (Wolfe-Simon et al., 2005). According to our results, the positively correlated FeSOD and AsA-POD activities of *S. maxima* reached their highest levels in control conditions on day 13 ($r = 0.829$) and they were 2.14 and 1.48 times higher than those of the supplemented conditions, respectively. As a cofactor of FeSOD and AsA-POD enzymes, the iron uptake of *S. maxima* increased up to day 13, showing coherence with the increases in activity of both enzymes. In addition, the highest FeSOD activity in *S. maxima* was 1.4-, 3.5-, and 4.4-fold higher than that reported from *Nostoc* sp. PCC 7120, *Synechococcus* sp. PCC 7942, and *Plectonema boryanum* UTEX 485 grown in BG-11 medium, respectively (Herbert et al., 1992; Regelsberger et al., 2004). As with *S. maxima*, FeSOD was considered to be the sole superoxide-scavenging enzyme in the S*ynechocystis* sp. strain PCC 6803 (Tichy & Vermaas, 1999). Accordingly, FeSOD could play a role in the dismutation of superoxide anion radicals, which are produced by the electron transport components of photosynthesis and respiration. The highest AsA-POD activity of *S. maxima* was 1.7 times higher than that of green algae *Chlorella vulgaris* Beijerink IAM C-27 grown in MC medium (Takeda et al., 1998). Because of its presence in all cell compartments and its high affinity to H_2O_2, it is well known that AsA-POD is an important enzyme participating in cell detoxification. AsA-POD removes H_2O_2 enzymatically, as well as non-enzymatically via AsA. In addition, the other antioxidant enzyme, CAT, which contains iron as a cofactor, was not determined in *S. maxima* using the nitrate concentrations. While the trend of increase in AsA-POD activity up to day 13 of incubation was observed, the content of AsA did not change significantly. This result may suggest that AsA content in the stress-dependent metabolism showed coherence with the enzyme activity. In the following period, decreases in AsA-POD activity and increases in AsA contents except for in the supplemented conditions also supported this idea. The highest AsA content in *S. maxima* was higher than that of cyanobacterium *P. boryanum* AUCC 143 (Prasad et al., 2005).

In general, the chlorophyll contents of cyanobacteria are affected by the cultivation medium components/conditions, cellular age, and light intensity. The highest chlorophyll content was obtained with intermediate light intensity, in agreement with Kebede and Ahlgren (1996). Therefore, at intermediate light intensities there is a balance between the energetic gains and chlorophyll biosynthesis. Depending on the incubation period, the chlorophyll contents of *S. maxima* grown in control conditions showed higher variation trends than those in the limited and supplemented nitrate conditions. This is in an agreement with the results of some previous studies (Piorreck et al., 1984; Gordillo et al., 1999). With respect to these results, nitrogen limiting in *S. maxima* resulted in the reduction of photosynthetic pigments. In photosynthetic organisms, stress factors such as nutrient limitation generally cause an imbalance in the electron transport system of photosystems, which are the first indication of unfavourable conditions (Turpin, 1991). In the present study, a correlation was also determined between the chlorophyll and magnesium levels up to day 13 of incubation ($r = 0.567$). Afterwards, the insignificant changes seen in the chlorophyll content may be associated with increasing magnesium levels.

The other antioxidant, phycocyanin, and total carotenoid contents increased with the increasing nitrate concentrations. However, these changes were determined to be insignificant in the control and nitrate supplemented conditions up to days 6 and 13, respectively. The highest phycocyanin content for the supplemented conditions was 1.7 and 1.2 times higher than that of the nitrate limited and control conditions, respectively. These data showed coherence with results obtained for *S. platensis* strain LB1475/a (Boussiba & Richmond, 1980). The highest value

is 5 times higher than that of *P. boryanum* (Prasad et al., 2005). It has been reported that phycocyanin also possesses certain therapeutic properties, such as antioxidant (including the scavenging of peroxynitrite and peroxyl radicals), anti-inflammatory, and hepaprotective properties (Romay et al., 2003). The highest total carotenoid contents observed were similar to that of cyanobacterium *P. boryanum* (Prasad et al., 2005). According to Abd El-Baky et al. (2004), the main carotenoid compounds found in *S. plantensis* are β-carotene, astaxanthine, lutein, and zeaxanthin. The accumulation of carotenoids occurred when microalgae *Dunaliella bardawil* UTEX 2538 cells were incubated under oxidative stress (Salguero et al., 2003).

In the presence of 10 and 30 mM concentrations of nitrate, the observation of insignificant changes in LPO levels in spite of decreases in FeSOD and AsA-POD activities after the day 13 may indicate that increases in total carotenoid and AsA contents and constant phycocyanin, chlorophyll, and iron levels are involved in protecting *S. maxima* membrane from damage. It is well known that AsA, as an antioxidant, scavenges the aqueous ROS by very rapid electron transfer that inhibits LPO (Halliwell & Gutteridge, 1989). A decrease in the LPO levels of *S. maxima* grown in supplemented nitrate conditions up to day 13 of incubation may demonstrate that the membrane is been sufficiently protected by increased AsA-POD activities, phycocyanin, total carotenoid and chlorophyll contents, and the lower iron and copper levels, which may cause decreasing Fenton-like reactions. Afterwards, increasing LPO levels may explain that the other mechanisms have been affected besides decreasing of AsA-POD activities and AsA contents. Similarly, it was reported that the AsA-POD activity of *Euglena gracilis* strain Z was reduced and the peroxidation of lipids and LPO levels were thus enhanced (Ishikawa et al., 1993).

In conclusion, *S. maxima* has good resistance to nitrate stress. FeSOD and AsA-POD activities, phycocyanin, and other produced pigments greatly enhance the resistance capability of this organism against nitrate. Enzymatic and non-enzymatic antioxidants of *S. maxima* have appreciable potential value as medicinal products and as additives for pharmaceutical, food, or cosmetic applications. Recently, *Spirulina* sp. has gained more attention because of its nutritional and various medicinal properties. Therefore, it could be used as a model for the biotechnological production of antioxidant compounds.

Acknowledgement

This study was supported by a project (2009. KB.FEN.038) from Dokuz Eylül University, Turkey.

References

Abd El-Baky HH, El Baz FK & El-Baroty GS (2004). Production of antioxidant by the green alga *Dunaliella salina*. *International Journal of Agriculture and Biology* 6: 49-57.

Benett A & Bogard L (1973). Complementary chromatic adaptation in filamentous blue-green algae *Spirulina platensis*. *Journal of Cell Biology* 58: 419-435.

Boussiba S & Richmond AE (1980). C-Phycocyanin as a storage protein in the blue-green algae *Spirulina platensis*. *Archives of Microbiology* 125: 143-147.

Boussiba S (1989). Ammonia uptake in the alkalophilic cyanobacteria *Spirulina platensis*. *Plant and Cell Physiology* 30: 303-308.

Bradford MM (1976). A rapid and sensitive method for quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72: 248-254.

Buege JA & Aust SD (1976). Lactoperoxidase-catalyzed lipid peroxidation of microsomal and artificial membranes. *Biochimica et Biophysica Acta: General Subjects* 444: 192-201.

Candan N & Tarhan L (2005). Effects of calcium stress on contents of chlorophyll and carotenoid, LPO levels, and antioxidant enzyme activities in *Mentha*. *Journal of Plant Nutrition* 28: 127-139.

Chadd HE, Newman J, Mann NH & Carr NG (1996). Identification of iron superoxide dismutase and a copper/zinc superoxide dismutase enzyme activity within the marine cyanobacterium *Synechococcus* sp. WH 7803. *FEMS Microbiology Letters* 138: 161-165.

Costa JAV, Kozza KL, Oliveira L & Magagnin G (2001). Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments. *World Journal of Microbiology and Biotechnology* 17: 439-442.

Crosti N, Servidei T, Bajer J & Serra A (1987). Modification of the 6-hydroxydopamine technique for the correct determination of superoxide dismutase. *Journal of Clinical Chemistry & Clinical Biochemistry* 25: 265-272.

Gordillo FJL, Jimenez C, Figueroa FL & Niell FX (1999). Effects of increased atmospheric CO_2 and N supply on photosynthesis, growth and cell composition of the cyanobacterium *Spirulina platensis (Arthrospira)*. *Journal of Applied Phycology* 10: 461-469.

Halliwell B & Gutteridge JMC (1989). Protection against oxidants in biological systems: The superoxide theory of oxygen toxicity. In: Halliwell B & Gutteridge JMC (eds.) *Free Radical in Biology and Medicine*, pp. 86-123. Oxford: Clarendon Press.

Herbert AK, Samson G, Fork DC & Laudenbach DE (1992). Characterization of damage to photosystems I and II in a cyanobacterium lacking detectable iron superoxide dismutase activity. *Proceedings of the National Academy of Sciences of the United States of America* 89: 8716-8720.

Ishikawa T, Takeda T, Shigeoka S, Hirayama O & Mitsunaga T (1993). Requirement for iron and its effect on ascorbate peroxidase in *Euglena gracilis*. *Plant Science* 93: 25-29.

Kebede E & Ahlgren G (1996). Optimum growth conditions and light utilization efficiency of *Spirulina platensis (=Arthrospira fusiformis) (Cyanophyta)* from Lake Chitu, Ethiopia. *Hydrobiology* 332: 99-109.

Kurilich AC, Tsau GJ, Brown A, Howard L, Klein BP, Jeffery EH, Kushad M, Wallig MA & Juvik JA (1999). Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry* 47(4): 1576-1581.

Latifi A, Ruiz M & Zhang CC (2009). Oxidative stress in cyanobacteria. *FEMS Microbiology Reviews* 33: 258-278.

Leduy A & Samson R (1982). Testing of an ammonia ion selective electrode for ammonia nitrogen measurement in the methanogenic sludge. *Biotechnology Letters* 4: 303-306.

Lichtenthaler HK & Wellburn AR (1983). Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. *Biochemical Society Transactions* 11: 591-592.

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science* 7: 405-410.

Moore LR, Post AF, Rocap G & Chisholm SW (2002). Utilization of different nitrogen sources by the marine cyanobacteria *Prochlorococcus* and *Synechococcus*. *Limnology and Oceanography* 47: 989-996.

Möller IM (2001). Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. *Annual Review of Plant Physiology* 52: 561-591.

Nakano Y & Asada K (1981). Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology* 22: 867-880.

Noctor G & Foyer CH (1998). Ascorbate and glutathione: keeping active oxygen under control. *Annual Review of Plant Physiology and Plant Molecular Biology* 49: 249-279.

Öztürk-Ürek R, Bozkaya LA & Tarhan L (2001). The effects of some antioxidant vitamin- and trace element-supplemented diets on activities of SOD, CAT, GSH-Px and LPO levels in chicken tissues. *Cell Biochemistry and Function* 19: 125-132.

Piorreck M, Baasch KH & Pohl P (1984). Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. *Phytochemistry* 23: 207-216.

Prasad SM, Kumar D & Zeeshan M (2005). Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to endosulfan stress. *Journal of General and Applied Microbiology* 51: 115-123.

Rai AK & Tiwari SP (2001). NO^{3-} nutrition and salt tolerance in the cyanobacterium *Anabaena* sp. PCC 7120 and mutant strains. *Journal of Applied Microbiology* 86: 991-998.

Regelsberger G, Laaha U, Dietmann D, Rüker F, Canini A, Grilli-Caiola M, Furtmüller PG, Jakopitsch C, Peschek AG & Obinger C (2004). The iron superoxide dismutase from the filamentous cyanobacterium *Nostoc* PCC 7120. *Journal of Biological Chemistry* 279: 44384-44393.

Romay CH, Gonzalez R, Ledon N, Remirez D & Rimbau V (2003). C-phycocyanin: A biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Current Protein & Peptide Science* 4: 207-216.

Salguero A, Benito M, Vigara J, Vega JM, Vilchez C & Leon R (2003). Carotenoids as protective response against oxidative damage in *Dunaliella bardawil*. *Biomolecular Engineering* 20: 249-253.

Takeda T, Yoshimura K, Ishikawa T & Shigeoka S (1998). Purification and characterization of ascorbate peroxidase in *Chlorella vulgaris*. *Biochimie* 80: 295-301.

Terzi R, Sağlam A, Kutlu N, Nar H & Kadıoğlu A (2010). Impact of soil drought stress on photochemical efficiency of photosystem II and antioxidant enzyme activities of *Phaseolus vulgaris* cultivars. *Turkish Journal of Botany* 34: 1-10.

Tichy M & Vermaas W (1999). In vivo role of catalase-peroxidase in S*ynechocystis* sp. strain PCC 6803. *Journal of Bacteriology* 181: 1875-1882.

Turpin DH (1991). Effects of inorganic N availability on algal photosynthesis and carbon metabolism. *Journal of Phycology* 27: 14-20.

Vonshak A (1997). *Spirulina platensis* (*Arthrospira*): *Physiology, Cell-Biology and Biotechnology*. London: Taylor & Francis.

Wolfe-Simon F, Grzebyk D, Schofield O & Falkowski PG (2005). The role and evolution of superoxide dismutase in algae. *Journal of Phycology* 41: 453-465.

Yang X, Wang X & Wei M (2010). Response of photosynthesis in the leaves of cucumber seedlings to light intensity and CO_2 concentration under nitrate stress. *Turkish Journal of Botany* 34: 303-310.

Zarrouk C (1966). *Contribution à l'étude d'une cyanophycée. Influence de divers facteurs physiques et chimiques sur la croissance et la photosynthèse de Spirulina maxima*. PhD dissertation, France: Université de Paris.